Effect of Solvent and Kinetic Study for Solid-Liquid Extraction of Bioactive Compounds from *Schizophyllum commune* and *Pycnoporus sanguineus*

Kinetic Study on Solid-Liquid Extraction Process

Yi Peng, Teoh[1], Mashitah, Mat Don*[2]

School of Chemical Engineering, Universiti Sains Malaysia,
14300 Nibong Tebal, Penang, Malaysia

[1]teoh.yipeng@gmail.com; *[2]chmashitah@eng.usm.my

Abstract

Studies on the effect of different types of solvent for the extraction of bioactive compounds from the white-rot fungi have been investigated. Results showed that 70% (v/v) methanol-water system provided the maximal extraction yield at 41.47% and 52.48% for *S. commune* and *P. sanguineus*, respectively. Fick's second law was used to calculate the predicted diffusion coefficient during the extraction process. For *S. commune*, the D_{fast} and D_{slow} obtained were 7.04 x 10^{-7} m^2/h and 8.42 x 10^{-8} m^2/h. This predicted data fitted well with the experimental data with R^2 less than 1. Flavonoid compound such as 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (DDMP) was detected in both strains using the gas chromatography-mass spectrometry (GC-MS).

Keywords

Schizophyllum Commune; Pycnoporus Sanguineus; Extraction; Yield; Kinetic

Introduction

Wood-decaying fungi were characterized either as brown rot and white rot (generally known as Basidiomycetes) or as soft rot (Ascomycetes and Fungi Imperfect) fungi (Teoh et al., 2011a). Also, wood-decaying fungi were a variety of fungi which were capable to digest wood, such as those attacked on dead wood called dry rot fungus (Ward et al., 2004); while those act as parasite on living trees called *Armillaria* or Honey fungus (Harding, 2008). *Schizophyllum commune* (*S. commune*) and *Pycnoporus sanguineus* (*P. sanguineus*) are normally associated with the white-rot of living trees and on logs, dead twigs, and stumps, leading to economic losses in forestry (Teoh et al., 2011b). *S. commune* is a species of basidiomycetes belonging to Schizophyllaceae of Agaricales (Teoh et al., 2012), while *P. sanguineus* belongs to the basidiomycetes of the family of Polyporaceae (Teoh et al., 2011b). Both of the fungi have been considered as potential sources of bioactive compounds particularly for its natural antifungal activity (Shittu et al., 2005; Fagade and Oyelade, 2009; Teoh et al., 2011b; Teoh et al., 2012).

Das et al. (2010) reported that phenols, phenolic acids, quinines, flavones, flavonoids, flavols, tannins and coumarins are commonly found in aromatic secondary metabolites from plants, in which these groups of compounds showed antimicrobial effect and served as plant defense mechanisms against pathogenic microorganisms. Flavones, flavonoids and flavonols had phenolic structure with one carbonyl group in order to give response to microbial infection and also effective antimicrobial substances against wide arrays of microorganisms (Das et al., 2010; Teoh et al., 2011b). Teoh et al. (2011b, 2012) in their work stated that 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-(DDMP) was found in the extracts of *S. commune* and *P. sanguineus*. This unique sugar residue belongs to the flavanoid fraction, a group of complex natural products of current medicinal interest, in which the anti-microbial, anti-oxidant, and anti-inflammatory properties have been recognized (Teoh et al., 2011b).

Solid-liquid extraction was an important stage of bioprocess isolation and identification of bioactive compounds from natural product for the economic production of biopharmaceuticals (Bucic-Kojic et al., 2007; Ly et al., 2007). A number of methods using organic or aqueous solvents were employed in the extraction of natural products, such as maceration,

percolation, soxhlet extraction, extraction under reflux, and steam distillation (Seidel, 2005). However, the soxhlet extraction method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (De Paira et al., 2004). The widely used extraction method was plant-tissue homogenization in solvent (Das et al., 2010), and the types of solvents used in the extraction process. A good solvent had several properties, such as low toxicity, ease of evaporation at lower temperature, promotion of rapid physiologic absorption of the extracts, preservative action and also the inability to cause the extract to complex or dissociate. The solvents that were commonly used for preliminary screening of antimicrobial activities from plants were methanol, ethanol and water (Ly et al., 2007; Parthasarathy et al., 2009; Das et al., 2010; Teoh et al., 2011b; Teoh et al., 2012).

Most of the extraction mechanisms were described using Fick's second law of diffusion, considering the mass transfer phenomenon (Cacace and Mazza, 2003; Bucic-Kojic et al., 2007; Ziaedini et al., 2010). However, less work has addressed the extraction process on fungal extracts with the aim of identifying and quantifying the influence of aforementioned variables on the mass transfer process in order to contribute to the process optimization, design and control of process and contributes to utilization of solvent. Moreover, this goal is particularly complicated in products with a complex geometry, such as fungal biomass.

In this study, solid-liquid extraction of bioactive compounds using different types of solvent from the white rot fungi *S. commune* and *P. sanguineus* was investigated. The kinetics and modeling of solid-liquid extraction of the fungus biomass were also highlighted.

Materials and Methods

Fungal Strain

The fungal strain, *S. commune* and *P. sanguineus*, were obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Malaysia. The stock culture was grown on malt extract agar (MEA) at 30°C and maintained on agar slants prior for subsequent studies.

Mycelia Suspension Preparation

Mycelia suspension was prepared by suspending mycelia discs from 7-d-old culture plates in sampling bottles containing sterilized distilled water, and 0.1% (v/v) Tween 80. The disc of 5 mm diameter was cut on the mycelia mats of the agar plate using a sterilized cork borer. A total of 10 discs for every 100 mL sterilized water was vortexed for 5 min in order to make the mycelia suspensions become homogenous.

Mycelia Extract Preparation

Ten milliliter (10% v/v) of the mycelia suspension was added to 90 mL of malt extract broth (containing 6.0 g/L malt extract, 1.2 g/L yeast extract, 1.8 g/L maltose, and 6.0 g/L dextrose in distilled water) in 250 mL Erlenmeyer flasks. The medium was autoclaved at 121°C for 15 min before the mycelia suspension was transferred into the culture media. The culture was incubated at 30±2°C, pH 6.5 in an incubator shaker at 200 rpm for 5 d. The culture broth was then harvested and centrifuged at 4000 × g for 15 min. The residues were then dried and homogenized prior to extraction process.

Extraction Process

A hydrodistillation process was carried out. Dried residues (100 g) obtained from the mycelia (biomass) were boiled in distilled water for 48 h in a ratio of 1 g : 20 mL. A similar procedure was carried out for the extraction using 70% (v/v) methanol-water and 70% (v/v) ethanol-water. Then, the percentage yield obtained was determined. The extraction yield was defined as the ratio of the amount of extractant obtained to the initial amount of solid mycelia used.

Mass Transfer Mechanism of Solid-Liquid Extraction

During the extraction process, the solvent, mycelia and bioactive compound were composed of the bulk liquid phase, solid phase and solute, respectively. In general, the solute was linked to the solid matrix by physical or chemical forces, in which it must be solubilized by the solvent first and then mass transfer resistance was overcome in order to transfer the bulk liquid phase (Ly et al., 2007; Ziaedini et al., 2010).

Since the extraction process was dynamic, thus it can be simplified into two parts according to the Fick's second law. In the first instance, it represented a period of constant and very rapid extraction rate (called washing period), where steady state mass transfer prevailed and the film resistance controlled the rate. While in the following steps, the process undergone a period of falling and much slower extraction rate

(called falling period), where unsteady state mass transfer prevailed and the intra-particle diffusion controlled the rate of process (Crank, 1975; Ziaedini et al., 2010).

Proposed mathematical Modeling using Fick's Second Law

In this study, the kinetic extraction data was fitted to the diffusion equation for mass transfer of solute from solid phase to liquid phase in stationary medium, assuming a flat plate shape for the dry residues. According to Fick's second law, the diffusion coefficients of solute can be predicted with the concentration change in the liquid phase with time by applying Eq (1).

$$\frac{\partial Ci}{\partial t} = D_i \frac{\partial^2 C_i}{\partial x^2} \quad (1)$$

where C_i denotes the concentration in the solid phase (g extractant/g mycelia), t is the time (s), D_i represents the diffusion coefficient (m^2/s), and x is the length (m).

The following assumptions were applied for this study:

- The dry residues are represented as flat plate with a thickness of 2*L*.
- The active compound is initially homogeneously contained in the solid mycelia.
- The active compound in the solid particle changes with time and distance.
- The concentration of active compound at the solid-liquid interface is in equilibrium.

The general solution of Eq (1) can be written as follows [13,16]:

$$\frac{C-C_0}{C_i-C_0} = 1 - \left[\frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\cos\frac{(2n+1)\pi x}{2L}\exp\left(-\frac{(2n+1)^2\pi^2}{4L^2}\right)Dt\right] \quad (2)$$

where C is the concentration at any given time at a distance x from the center within the plate coordinate ($x = \pm L$).

Next, the mass transfer from the plate (M) can be calculated by integrating the concentration over the thickness. Eq (3) represented the mass transferred at time t relative to the total amount transferred after infinite time (M_∞).

$$\frac{M}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{n=\infty}\frac{1}{(2n+1)}exp\left(-\frac{(2n+1)^2\pi^2}{4L^2}Dt\right) \quad (3)$$

According to Ziaedini et al. (2010), all terms except the first became negligible after a short period of extraction process. This phenomenon was due the fact that it is necessary to consider the presence of two parallel diffusion processes inside the solid phase, particularly the fast and slow step. Thus, Eq (3) can be rewritten as follows:

$$\left(\frac{C_\infty - C}{C_\infty}\right) = \frac{8}{\pi^2}\left[f_1 exp\left(-\frac{\pi^2 D_1 t}{4L^2}\right) + f_2 exp\left(-\frac{\pi^2 D_2 t}{4L^2}\right)\right] \quad (4)$$

where f_1 and f_2 are the fractions of the solute which are extracted with diffusion coefficients D_1 and D_2, respectively.

During the earlier stage of extraction (washing period), the second exponential term (Eq 4) approached to unity and D_1 and f_1 can be determined. On the other hand, the second term remained significant in the later stage of extraction (falling period). Thus a plot of the *ln* function against time can be drawn, and thus providing D_2 from the slope and f_2 from the intercept. The predicted model was then validated using determination coefficient (R^2). According to Nagelkerke (1991), when the R^2 value was closed to 1, then the predicted data fitted well to the proposed model.

Gas Chromatography-Mass Spectrometry Analysis

The crude extract was dissolved with 70% (v/v) ethanol prior to the gas chromatography-mass spectrometry analysis. In this study, a GC-MS was used to analyze the sample quantitatively, by referring to the molecular weight of the compounds in a library (Model: NIST), that was incorporated into it. The gas chromatography analyses were performed using a Perkin Elmer Clarus 600 gas chromatograph equipped with an ELITE-5 column. The gas chromatography was coupled to the Perkin Elmer 600T mass spectrometer. The oven temperature was programmed at 65°C for 4 min and then increased to 280°C at a rate of 8°C/min.

Results and Discussion

Effect of Different Types of Solvent

The effect of different types of solvent on the extraction kinetics of active compounds from *S. commune* and *P. sanguineus* were examined using 70% (v/v) methanol-water, 70% (v/v) ethanol-water and distilled water. In order to make the data more accurate and reliable, the results were expressed on dry basis, and each experimental run was carried out in triplicates.

Figure 1 demonstrated the extraction yield obtained from *S. commune* using 70% (v/v) methanol-water, 70% (v/v) ethanol-water and distilled water as solvents for a time period of 48 h of extraction. The equilibrium was not reached until the extraction time was 24 h. Once the liquid phase had achieved its equilibrium concentration, the concentration gradient became zero, and hence no further extraction was achievable. The

extraction yield was found to be the highest (0.7092%) when the biomass was extracted using 70% (v/v) methanol-water. Similar pattern was also observed on the extraction profiles of active compounds by *P. sanguineus* (Figure 2). The result showed that the extraction rate rapidly increased in the first 20 h; then it reached equilibrium and became stabilized. The amount of active compound extracted using 70% (v/v) methanol-water system was about 0.5053%, in which it was 28.75% lower than that obtained from *S. commune*.

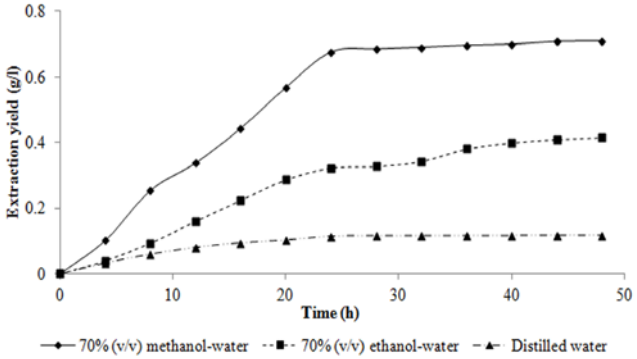

FIGURE 1 COMPARISON OF THE EXTRACTION PROFILES FROM *S. commune* USING 70% (v/v) METHANOL-WATER, 70% (v/v) ETHANOL-WATER, AND DISTILLED WATER FOR A TIME PERIOD OF 48 h OF EXTRACTION

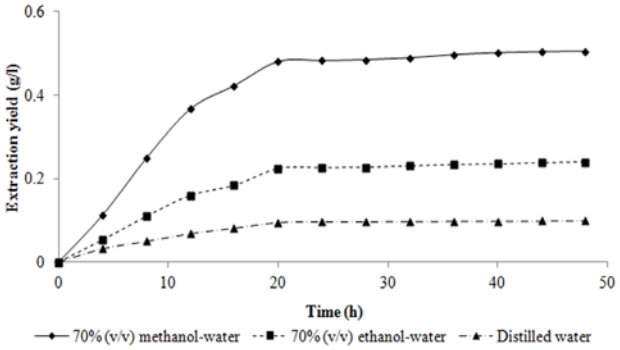

FIGURE 2 COMPARISON OF THE EXTRACTION PROFILE FROM *P. sanguineus* USING 70% (v/v) METHANOL-WATER, 70% (v/v) ETHANOL-WATER, AND DISTILLED WATER FOR A TIME PERIOD OF 48 h OF EXTRACTION

Zjawiony (2004) stated that the water extracts of the basidiocarps from aphyllophorales (polypore) fungi contained predominantly polysaccharides, being the principal components of the fungal cell wall, and these higher molecular weight compounds were capable to provide the immunoprotective activities. Hence, all extraction systems in this present study used water as solvent since the active compounds extracted were used as antifungal agent. As it can be seen in Figure 1 and 2, the extraction processes that were carried out using 70% (v/v) methanol-water and 70% (v/v) ethanol-water system gave better yield as compared to those of distilled water system only. This phenomenon was due to the fact that the alcoholic extract provided a more complete extraction as compared to the distilled water extraction system in order to produce less polar compounds, which could possess wider spectrum for antimicrobial activities (Parthasarathy et al., 2009; Teoh et al., 2011b; Teoh et al., 2012). In addition, it was found that the extract obtained from organic solvent (methanol and ethanol) gave more consistent antimicrobial activities as compared to those from water extract (Das et al., 2010). The 70% (v/v) methanol-water system gave higher yield for both extraction processes using *S. commune* and *P. sanguineus*. This could be due to that methanol is a strong solvent and exhibited as the smallest alcoholic molecule, which was able to undergone more complete extraction reaction as compared to the ethanol extraction (Ly et al., 2007).

Kinetics and Modeling

Figure 3 showed a typical corresponding plot of the function *ln* $((C_\infty - C)/C_\infty)$ against time for the extracted *S. commune* and *P. sanguineus* using 70% (v/v) methanol-water system. This first order plot was then used to determine the mass transfer rate for the fast stage as well as for the later slower second stage of extraction by applying Eq (4), and summarized in Table 1.

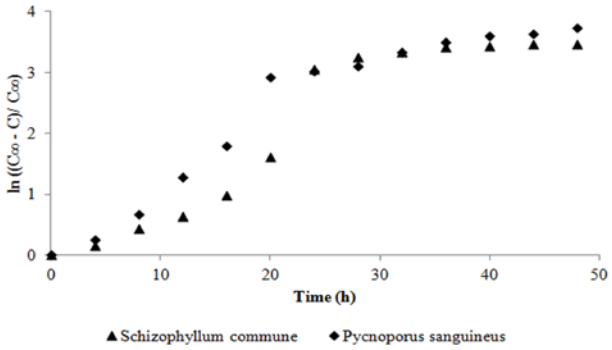

FIGURE 3 TYPICAL CORRESPONDING PLOT OF THE FUNCTION ln $((C_\infty - C)/ C_\infty)$ AGAINST TIME FOR THE EXTRACTED *S. commune* and *P. sanguineus* using 70% (v/v) METHANOL-WATER SYSTEM

TABLE 1 DIFFUSION COEFFICIENTS (D_{fast}, D_{slow}) FOR EXTRACTION OF BIOACTIVE COMPOUNDS FROM *S. commune* and *P. sanguineus* USING THREE TYPE OF SOLVENT IN HYDRODISTILLATION PROCESS

Type of Solvent	*Schizophyllum commune*			*Pycnoporus sanguineus*		
	D_{fast} (m^2/h)	D_{slow} (m^2/h)	R^2	D_{fast} (m^2/h)	D_{slow} (m^2/h)	R^2
70%(v/v) methanol-water	7.04 x 10^{-7}	8.42 x 10^{-8}	0.998	6.33 x 10^{-7}	8.64 x 10^{-7}	0.997
70%(v/v) ethanol-water	1.20 x 10^{-7}	6.60 x 10^{-8}	0.992	6.32 x 10^{-7}	9.41 x 10^{-8}	0.993
Distilled water	2.42 x 10^{-8}	4.29 x 10^{-10}	0.981	4.29 x 10^{-7}	3.74 x 10^{-8}	0.984

It can be observed that the D_{fast} during 70% (v/v) methanol-water extraction system was the fastest as compared to other solvent used. For *S. commune*, the highest diffusion coefficient was 7.04 x 10^{-7} m^2/h with R^2=0.998. By using 70% (v/v) ethanol-water and distilled water extraction system, the diffusion coefficients gradually decreased by 82.95% and 96.56%, respectively. Similar trends were also observed for the D_{slow}, with 70% (v/v) methanol-water extraction system being the highest at 8.64 x 10^{-8} m^2/h.

GC-MS Analysis

Figure 4(a) and 4(b) showed the GCMS spectrum obtained from the *S. commune* and *P. sanguineus* methanol extracts, respectively. In this study, the bioactive compound 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (DDMP) which belongs to the flavonoid compound was observed. The retention time for this compound in both strains was 8.19 min.

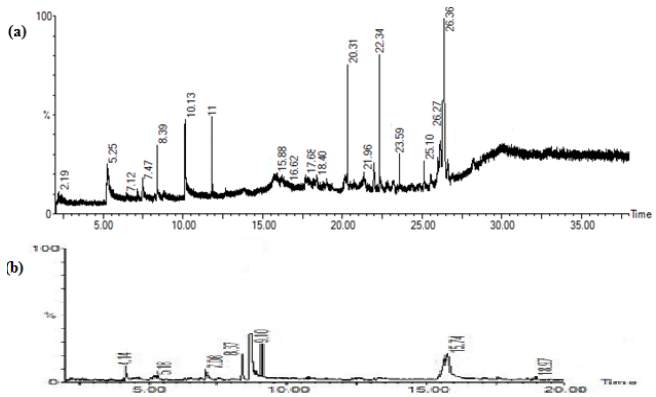

FIGURE 4 GC-MS CHROMATOGRAM FOR (a) *S. commune* and (b) *P. sanguineus* methanol extracts

Conclusion

The maximum yield for the extraction of bioactive compounds from white-rot fungi, *S. commune* and *P. sanguineus*, were attained using 70% (v/v) methanol-water. For the kinetic study, two stages of extraction processes which were fast stage and slow stage were detected. The diffusion coefficients, D_{fast} and D_{slow}, corresponding to the diffusion occurred in shorter and longer time period, respectively. The D_{fast} for both extraction processes varied from 7.04 x 10^{-7} to 2.42 x 10^{-8} m^2/h using 70% (v/v) methanol-water, 70% (v/v) ethanol-water and distilled water as solvents. The compound 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (DDMP) was detected in both extractants, in which it was a flavonoid that can be served as bioactive compound for various biopharmaceuticals products.

ACKNOWLEDGMENT

The authors gratefully acknowledge the National Science Fellowship (NSF) from the Ministry of Science, Technology and Innovation (MOSTI) of Malaysia and the Research University (RU) grant (Grant Account No.: 814057) provided by University Sains Malaysia to support this research work.

REFERENCES

Bucic-Kojic, A., Planinic, M., Tomas, S., Bilic, M., and Velic, D. "Study of solid-liquid extraction kinetics of total polyphenols from grape seeds," Journal of Food Engineering 81 (2007): 236-242.

Cacace, J. E., and Mazza, G., "Mass transfer process during extraction of phenolic compounds from milled berries," Journal of Food Engineering 59 (2003): 379-389.

Crank, J. The Mathematics of Diffusion, 2nd ed. New York: Oxford University Press, 1975.

Das, K., Tiwari, R. K. S., and Shrivastava, D. K. "Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends," Journal of Medicinal Plants Research 4 (2010): 104-111.

De Paira, S. R., Lima, L. A., Figueiredo, M. R., and C. Kaplan, M. A. "Plumbagin quantification in roots of *Plumbago scandens* L. obtained by different extraction techniques," Anais da Academia Brasileira de Ciencias 76 (2004): 499-504.

Fagade, O. E., and Oyelade, A. A. "A comparative study of the antibacterial activities of some wood-decay fungi to synthetic antibiotic discs," Electronic Journal of Environmental, Agricultural and Food Chemistry 8 (2009): 184-188.

Harding, P. Collins Mushroom Miscellany. USA: Collins, 2008.

Ly, M., Margaritis, A., and Jajuee, B. "Effect of solvent concentration on the extraction kinetics and diffusivity of Cyclosporin A in the fungus *Tolypocldium inflatum*," Biotechnology and Bioengineering 96 (2007): 67-79.

Nagelkerke, N. J. D. "A note on a general definition of the coefficient of determination," Biometrika 78 (1991): 691-692.

Parthasarathy, S., Azizi, J. B., Ramanathan, S., Ismail, S., Sasidharan, S., Said, M. I. M., and Mansor, S. M. "Evaluation of antioxidant and antibacterial activities of

aqueous, methanolic and alkaloid extracts from *Mitragyna speciosa* (Rubuaceae family) leaves," Molecules 14 (2009): 3964-3974.

Seidel, V. "Chapter 2 - Initial and bulk extraction," In: Natural Products Isolation, 2nd ed., edited by Sarker, Z. Latif, S. D., Gray, A. I., 27-45, USA: Humana Press Inc., 2005.

Shittu, O. B., Alofe, F. V., Onawunmi, G. O., Ogundaini, A. O., and Tiwalade, T. A. "Mycelial growth and antibacterial metabolite production by wild mushrooms," African Journal of Biomedical Research 8 (2005): 157-162.

Teoh Y. P., Mashitah, M. D., and Ujang, S. "Assessment of the properties, utilization, and preservation of rubberwood (*Hevea brasiliensis*): A case study in Malaysia.," Journal of Wood Science 57 (2011a): 255-266.

Teoh, Y. P., Mashitah, M. D., and Ujang, S. "Media selection for mycelia growth, and antifungal activity against wood-degrading fungi, and GC-MS study by *Pycnoporus sanguineus*," Bioresources 6 (2011b): 2719-2731.

Teoh, Y. P., Mashitah, M. D., and Ujang, S. "Nutrient improvement using statistical optimization for growth of *Schizophyllum commune*, and its antifungal activity against wood degrading fungi of rubberwood," Biotechnology Progress 28 (2012): 232-241.

Ward, G., Hadar, Y., and Dosoretz, C. G. "The biodegradation of lignocelluloses by white rot fungi," In: Fungal Biotechnology in Agricultural, Food, and Environmental Applications, edited by Arora, D. K., 393-406, UK: Marcel Dekker Inc., 2004.

Ziaedini, A., Jafari, A., and Zakeri, A. "Extraction of antioxidants and caffeine from green tea (*Camelia sinesis*) leaves: Kinetics and modeling," Food Science and Technology International 16 (2010): 505-506.

Zjawiony, J. K. "Biologically active compounds from Aphyllophorales (polypore) fungi," Journal of Natural Products 67 (2004): 300-310.